UNIVERSITY OF PENNSYLVANIA

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A THESIS

IN BOTANY

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RESISTANCE OF PINUS SYLVESTRIS TO A GALL-FORMING PERIDERMIUM[1]

W. G. HUTCHINSON[2]

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INTRODUCTION

In 1926, York (43) reported that a gall-forming Peridermium was causing considerable injury to Scotch pine, *Pinus sylvestris* L., at the Masonic Summer Camp on Round Lake in the vicinity of Woodgate, New York. It has yet not been definitely determined[3] whether this is a new species of Peridermium, some atypical, autoecious form of *Cronartium quercuum* (Berk) Miyabe (*C. cerebrum*), or one of the western-United States gall rusts. Growing among the heavily infected trees in this Woodgate area are many trees that, although artificially inoculated for 6 successive years, have shown no gall formation. The present investigation deals with this phenomenon of resistance as studied from the standpoints of gross morphology, histology, and physiology.

LITERATURE REVIEW

There is a marked paucity of literature, other than observational accounts, on the resistance of forest trees to fungus disease; in fact, there are few recorded attempts to study the basis of resistance in woody plants of any kind. Most discussions relative to the resistance of plants to the invasions of parasites point out that resistance may have a morphological or a physiological basis or a combination of the two. The formation in the host tissue of a definite layer of cork cells in advance of the invading mycelium has been considered by several investigators to represent an attempt to check the spread of the parasite. Conant (9), in studying the cause of resistance of certain strains of tobacco to *Thielavia basicola* (B.

[1] A thesis submitted to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] The writer is indebted to Dr. H. H. York for his helpful advice and criticism during the progress of this work.

[3] In the recently published Manual of the Rusts in United States and Canada p. 24, Arthur states "Another rust on *P. sylvestris* . . . recently discovered in northern New York and called 'Woodgate Rust'" is "now referred to *C. quercuum.*"

and Br.) Zopf, recorded that such corky areas between diseased and healthy tissues successfully prevented the spread of the fungus through the plant. Other investigators have stated that the resistant character of cell walls may account for protection against disease. Melander and Craigie (26) found that varieties of Berberis immune from *Puccinia graminis* Pers. were provided with thicker cuticles and with cell walls more resistant to mechanical puncture than were those susceptible to infection. In many instances the products of cell metabolism and their physical and chemical relationships have been considered to have a very direct bearing upon resistance and susceptibility. Dufrénoy (14), from a study of *Blepharospora cambivora* Petri on chestnut, *Phytophthora infestans* (Mont.) de Bary on potato, and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. on bean, has concluded that the accumulation of phenolic compounds in vacuoles represents a local immunity in the plant. Newton, Lehmann, and Clarke (29) have suggested a relation between the resistance of wheat to *Puccinia graminis tritici* Eriks. and Henn. and the phenolic compounds present in the plants. Cook and his associates (10, 11) have shown that tannin may exert an inhibitory action upon the growth of many species of fungi in culture. A careful biochemical study by Angell, Walker, and Link (4) has recently shown that resistance of onions to onion smudge is definitely related to the presence of protocatechuic acid in the outer pigmented scales. Dickson, Link, and Dickson (12) have found in the case of seedling blight that resistant corn seedlings contained 20 per cent more uronic acids than susceptible seedlings. Ezekiel (16), in an extensive study of resistance of wheat to *Puccinia graminis tritici*, has recorded a correlation between resistance and the growth and branching of germ tubes of the spores in extracts from resistant and susceptible plants. Thomas (36) reports that, although the growth of *Armillaria mellea* (Vahl) Quel. is inhibited by the extractives of the roots of certain trees, there apparently is no relationship to resistance. It is conceivable that the cell sap of certain plants may be uncongenial for fungus growth because of its lack or excess of hydrogen ions. Many experimental attempts have been made to demonstrate such a relationship. Comes (8) has found that a rust-resistant strain of wheat has a greater acidity than susceptible forms. When this same strain, however, is grown under different environmental conditions it becomes susceptible and its cell sap then shows a reduced acidity. On the other hand, many investigators have failed to find any such relationship. Hurd (20), as well as Newton, Lehmann, and Clarke (29), could demonstrate no relation between the pH of cell sap and the resistance of wheat to *Puccinia graminis tritici*. Harris and his coworkers (19) have clearly shown that the osmotic concentration and electrical conductivity of sap expressed from phanerogamic parasites are greater than

those from the host plants. Similar work has been done to determine whether these physical values were any different in resistant and susceptible plants. Newton, Lehmann, and Clarke (29) have been unable to demonstrate any important difference between tissue fluids of wheat plants resistant and susceptible to *Puccinia graminis tritici* as evidenced by osmotic pressure or electrical conductivity.

In addition to the factors considered above, a number of investigators have found that in plants subjected to infection, especially by rusts, there may be a type of reaction referred to as "protoplasmic resistance" or as "hypersensitiveness." This phenomenon was first observed and studied by Ward (37, 38, 39). He noted that in resistant plants hyphae were formed and haustoria penetrated host cells, but that such cells died soon after invasion, and that the hyphae and haustoria degenerated and appeared like experimentally starved mycelium. He attributed this reaction primarily to starvation of an obligate parasite. Marryat (25) studied infection by *Puccinia glumarum* Eriks. & Henn. in both resistant and susceptible strains of wheat. She also considered that resistance was brought about by the death of host tissue resulting in the death of the parasite from starvation. She further suggested that there might be a formation of toxin and antitoxin perhaps mutually destructive. Gibson (18) studied the early growth of various rusts that had infected plants other than their natural hosts. She rejected the starvation hypothesis of Ward, and considered that toxic substances formed by the host cells might be responsible for the failure of the hyphae to form haustoria and for the ultimate death of the mycelium. In natural hosts that were resistant, she found haustoria present, but the mycelium had degenerated after a few days. This phenomenon of hypersensitiveness in wheat infected with rusts also was studied by Stakman (33). He found in susceptible plants a ready adjustment of parasite and host, but an intolerant relationship in resistant plants. The rust hyphae, although forming few haustoria in resistant plants, brought about the death of host cells adjacent to them. They continued to grow for a time and to affect other cells, but soon perished. In some cases haustoria continued to live after the death of the cells that they had penetrated. He, therefore, concluded that the reaction is not due primarily to starvation but to a toxin-antitoxin relationship. Mains (24), investigating infection in strains of corn resistant and susceptible to *Puccinia sorghi* Schw., suggested that the normal symbiosis among cells of the plants is destroyed by the action of the fungus, causing some of them to parasitize others. He considered carbohydrates to play an important rôle in this resistance phenomenon. Allen (1, 2, 3), in a detailed cytological study of wheat resistant to *Puccinia graminis tritici,* has regarded Ward's theory of starvation as "untenable." She has suggested that an antagonistic relationship obtains

in the cell in that the cell is first killed by toxin produced by the haustorium, which in turn is killed by the products of cell degeneration.

The relation of environmental factors to susceptibility and resistance has received much attention. Eckholt (15) is inclined to consider environmental factors, such as the physical and chemical nature of the soil, soil moisture, and soil temperature, of more importance in infection of wheat with *Puccinia glumarum* than hereditary factors of resistance and susceptibility. In an extensive study of wheat grown in nutrient solutions, Doak (13) has recently found that an excess of potassium or lack of nitrogen increases resistance to *Puccinia triticina* Eriks.

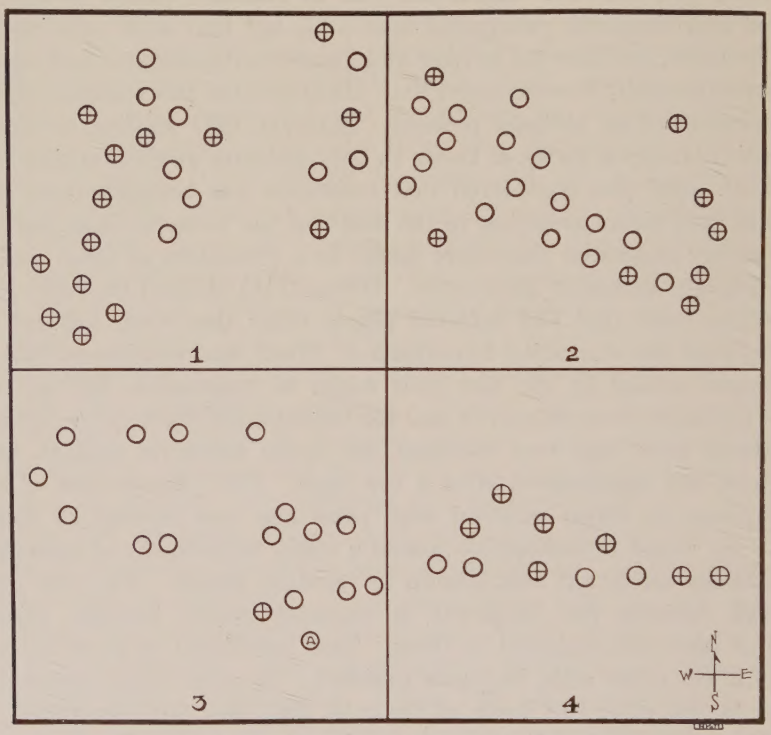

FIG. 1. Diagram of plots of Scotch pine trees. ○, resistant; ⊕, susceptible; A, location of parent tree.

METHODS

Description of plots. In the early summer of 1930, investigations were begun on 4 plots of *Pinus sylvestris* at Woodgate, N. Y. (Fig. 1). Some of the trees in these plots had been inoculated previously by H. H. York. In plot 1, resistant trees of varying age and height are growing near susceptible trees of about the same age and height. Plot 2 consists of

a few trees 8 to 10 years old. About half of these are resistant and half susceptible. In plot 3, there are several trees 10 to 12 years old, all of which are apparently seedlings from one tree, the position of which is indicated by the letter A. This tree, although exposed to natural infection, never developed galls, but succumbed to Armillaria infection. Every tree in this plot except one shows marked resistance. Plot 4 consists of numerous resistant trees about 20 years old growing near groups of susceptible trees.

Inoculations. For 3 consecutive seasons, resistant and susceptible trees have been artificially inoculated. Quantities of galls with mature aecia have been collected in June and masses of aeciospores scraped from them. The terminal portions of branches with the current season's growth were heavily painted with suspensions of these spores in water. The iceless refrigerator described by York[4] was used as a protection about these inoculated branches. This chamber was removed at the end of 48 hours.

Collections have been made from such inoculated branches at intervals varying from 48 hours to 6 years after inoculation. Similar collections have been made from noninoculated trees growing at a distance of 15 miles from the site of the plots, and also a few collections from healthy trees at the Arnold Arboretum, Jamaica Plain, Massachusetts.

Preparation of Tissues. In the preparation of tissues for sectioning several killing and fixing solutions have been used. Formalin alcohol (4 parts formalin to 100 parts 70 per cent alcohol), Carnoy's solution, and Schaffner's chromo-acetic-acid solution have given particular success. Bouin's solution, picro-formol, Fleming's fluid, and Conant's solution also have been used with success.

Some of the tissues were imbedded in nitrocellulose according to the technique described by Jeffrey (21) and Wetmore (41), excellent sections being obtained by this method. In order to obtain serial sections, however, many tissues were imbedded in paraffin by the butyl-alcohol method described by Zirkle (45). This procedure has been found invaluable for the cutting of 1- and 2-year-old stems. Sections, whether cut on the rotary or sliding microtome, were cut from 8 to 15 microns thick. Transverse sections have been used largely, but in many instances radial and tangential sections also.

Staining. The sections were stained with several combination stains. Those intended primarily for the study of normal or abnormal histology usually were stained with the safranin-haematoxylin combination or Fleming's triple stain (6). When it was desired to study the sections for the presence of mycelium and to trace this mycelium through and between cell walls, the combination of orseillin and anilin blue described by Strasburger (35) was used with excellent results.

[4] York, H. H. Celluloid "iceless refrigerator" and some results from field inoculations with the aeciospores of Woodgate rust (*Peridermium* sp.). In manuscript.

Microchemical tests. In connection with the histological study of sections prepared by imbedding, a series of microchemical tests was made on sections freshly cut on the ether-freezing microtome. In most cases the tissues were sectioned a few minutes after they were removed from the tree. Starch, tannin, fats, resin, gums, cellulose, suberin, cutin, lignin, and pectin were identified by means of tests outlined by Eckerson[5] or Stevens (34).

Physiological studies. Quantitative chemical analyses have been made of young twigs from both resistant and susceptible trees with regard to tannin, calcium, and potassium content. Analyses of various samples of soil collected at the base of both resistant and susceptible trees have been made with reference to the quantitative determination of calcium and potassium.

Juices expressed from the twigs of resistant and susceptible trees by pulverizing the ground twigs with liquid air and subjecting this frozen mass to a pressure of 200 pounds per square inch for 10 minutes have been used for determination of osmotic pressure and hydrogen-ion concentration.

An attempt has been made to reproduce in the resistant twigs the pathological changes found following infection. Extractives prepared from galls and from germinating spores were introduced into the tissues through small capillary tubes and also by hypodermic injection. Oxalic acid has been used in the same way, several investigators having reported a relationship of this acid to the pathogenicity of fungi (5, 22).

FIELD OBSERVATIONS OF HOST REACTIONS

In their response to infection the inoculated trees have shown many different reactions ranging from highly susceptible to resistant. For the purposes of this discussion, however, 3 principal types of reaction may be distinguished. Type A occurs only in susceptible trees. During the first growing season there may be slight evidence of infection other than small brownish spots followed by minute swellings. The following spring, however, marked hypertrophies develop and typical galls soon form (Fig. 2, A). In the reaction here designated as type B, there is often definite evidence of infection a few months after inoculation. Isolated discolored areas become confluent and for several inches along the twigs there will be reddish-brown discolorations. During the following growing season an excessive splitting of bark takes place on these twigs, sometimes accompanied by a slight resinosis (Fig. 2, B). Occasionally a few abortive or atypical galls may develop from such areas. In type C reaction numerous small spots may be noted a few weeks after inoculation (Fig. 2, C). These are often greenish brown or slightly reddish in the center, sometimes sur-

[5] Eckerson, S. H. Microchemistry. University of Wisconsin. (Mimeographed).

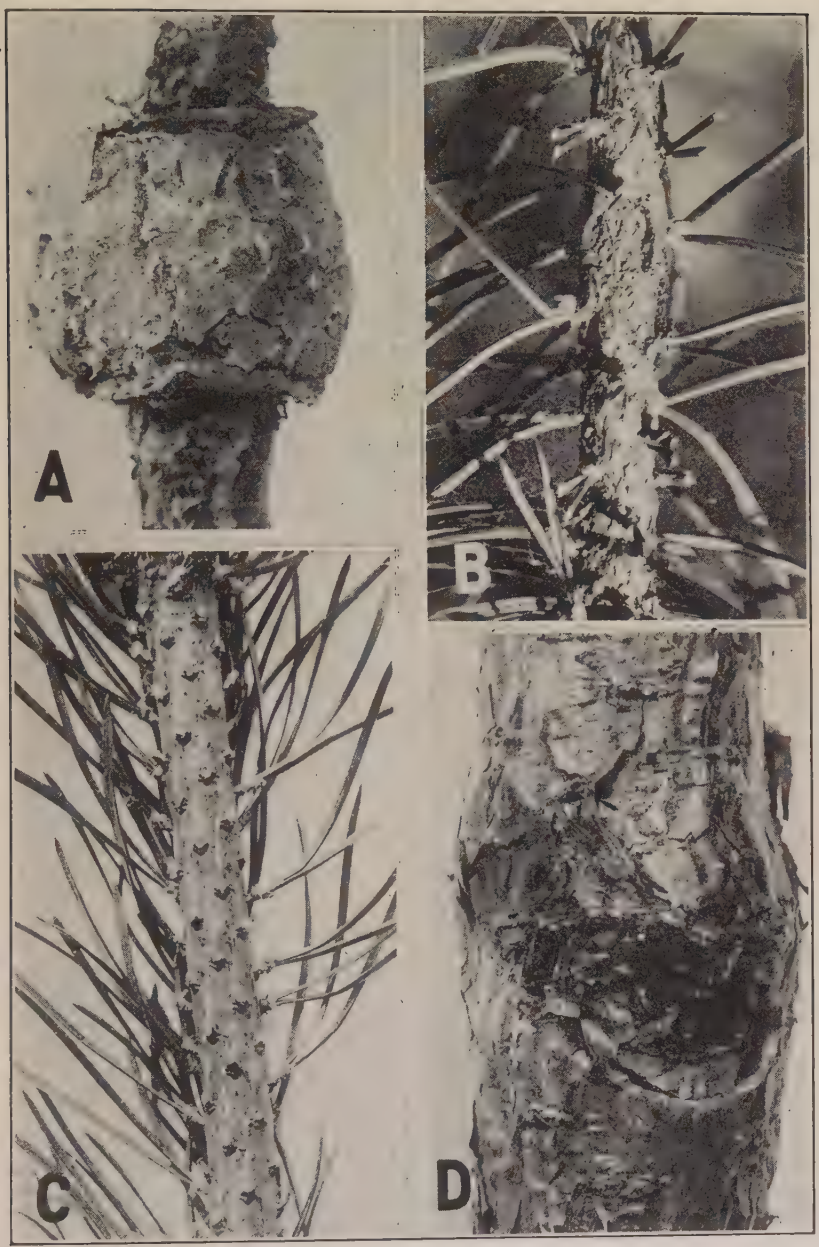

FIG. 2. A. Twig of susceptible Scotch pine 3 years after inoculation showing gall typical of type A reaction. B. Partially resistant twig 1 year after inoculation showing cracked bark characteristic of type B reaction. C. Resistant twig 3 months after inoculation showing spotted areas commonly found in type C reaction. D. Resistant twig 3 years after inoculation showing exfoliation of bark often found in type C reaction. Photographs by H. H. York.

rounded by a lighter chlorotic halo. Similar infection spots have been found developing on various resistant species of pine artificially inoculated with this rust (28, p. 199). During the following growing season, it is not uncommon to find a few swellings developed on these twigs as though galls were to be produced. These swellings rarely increase in size during the third year, but, through the growth of the surrounding tissue, they gradually become indistinct. During the third or fourth year, there can be noted at the position of these swellings a marked exfoliation of bark and sometimes a slight atrophy (Fig. 2, D).

It should be pointed out here that the different reactions described above are not always definitely segregated to certain trees. For instance, types B and C both may be found associated with type A on susceptible trees. Type C, and occasionally type A, may be associated with type B on what we may call partially resistant trees. However, type C only is found on the truly resistant trees. This point will be considered later in connection with the interpretation of the resistant reactions.

Perhaps a brief explanation should be given in connection with the terms "resistant," "partially resistant," and "susceptible," as used above. Gall formation is used as a criterion of susceptibility, since it is only those trees producing galls that show any visible signs of injury. Those trees may be considered partially resistant in which the majority of infection centers are prevented from forming galls. The trees that, although they become infected, are able in some way to check all infection centers and produce no galls can be considered resistant.

HISTOLOGICAL STUDIES

A comparative study of serial sections of noninfected twigs of both susceptible and resistant trees with reference to size of cells and thickness of cell walls has shown no outstanding difference. A microchemical study made of such twigs has shown no significant difference in regard to food storage or chemical character of cell walls. There is considerable variation in the amounts of tannin and fat, but these substances also vary widely among twigs of the same tree.

The Normal Twig

This brief description of the normal twig will include only those structures that have a bearing on penetration and spread of the fungus. The description applies equally to resistant and susceptible twigs during the period when they are subject to infection.

The single layer of epidermal cells is covered over by a cuticle averaging 3 μ in thickness. The cortex consists of 10 to 20 cell layers, the outer 4 or 5 of which rarely store starch during the season when infection might

occur, although the other cell layers may store large quantities. Starch, often in association with fats and resins, also may be stored in the pith, medullary and xylem rays, and phloem parenchyma. Tannin, for the most part, is confined to isolated cells or groups of cells in the cortex, phloem parenchyma, and pith. It also may be found in and near the cambium and in the vicinity of leaf traces. A well-developed periderm may be distinguished during the first growing season. The phellem cells give tests for suberin when tested with ammoniacal gentian violet. A continuous layer of sclerenchyma forms directly internal to the periderm. These cells show a positive lignin reaction. Usually the 3 layers of xylem elements nearest the cambium are free from lignin.

Penetration

The usual method of entrance of the fungus into the host is through the young twigs (44). The germ tube of the spore penetrates the cuticle of susceptible and resistant trees with apparently the same ease (Fig. 3, F and G). No evidence has been secured to show whether the method of penetration, is enzymatic or mechanical.

Growth of Mycelium and Host Reactions

Reaction type A. In a susceptible host, the mycelium is frequently intercellular, forming few or no haustoria for as much as the first month of its development. The cortex cells adjacent to the advancing mycelium become abnormally granular and stain more heavily than normal cells of the same kind. From microchemical tests it can be shown that these cells contain an abundance of tannin, while those more recently surrounded by mycelium are more nearly normal (Fig. 4, B). These latter cells, even at a later stage, do not become so densely granular as do those first attacked. It appears that the cells gradually lose their ability to produce or acquire tannins or else the necessity for tannin formation is withdrawn. It will be noted in figure 4, B, that the haustoria are few and decidedly stunted.

As the mycelium continues to spread through the cortex, the nutritional relationship seems to change, for many large haustoria are formed. The hypha becomes very much constricted as it passes through the minute opening that it produces in a cell wall to form a haustorium (Fig. 3, A). The host cells thus parasitized appear in no way to depart from their normal condition. We have here, then, an example of the almost mutualistic relationship of host and parasite so often reported in the literature, especially in the case of rusts (3, 24, 25, 37, 38, 40).

The early reaction to infection is not always like that just described. Sometimes, in the susceptible host the mycelium forms large haustoria

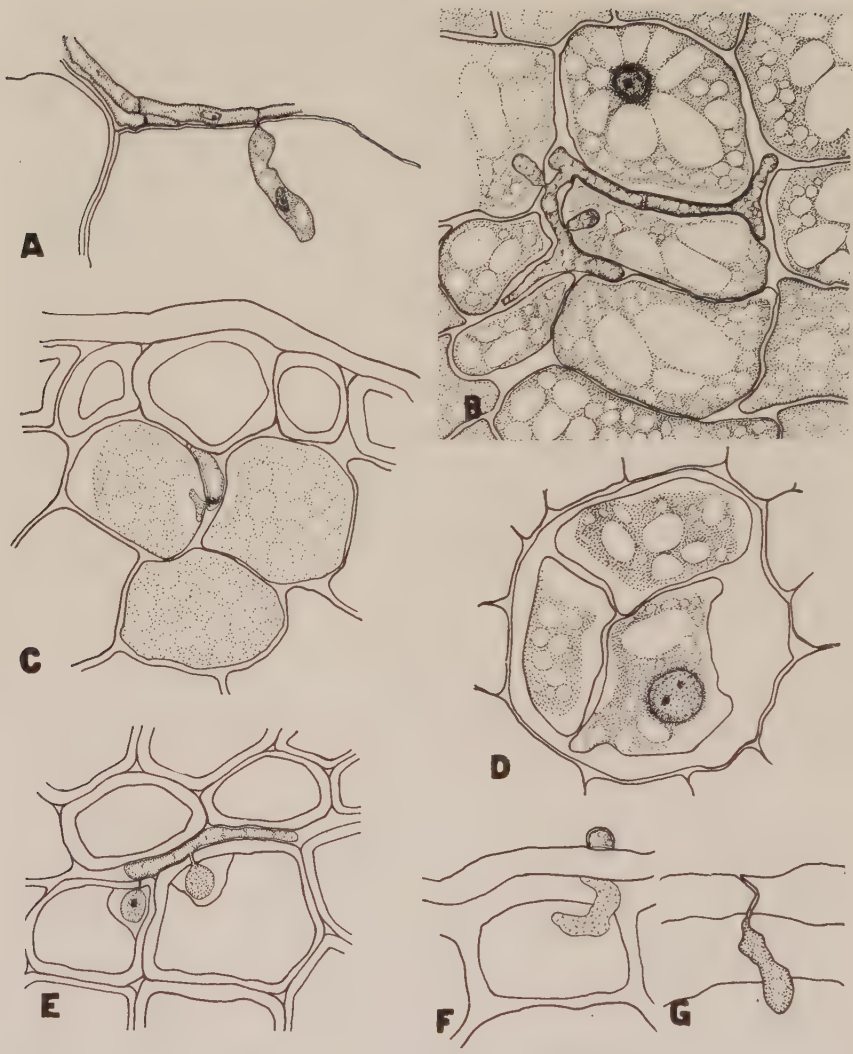

FIG. 3. A. Portion of cortical cell of susceptible host showing mature haustorium. ×1000. B. Portion of cortical region of susceptible host showing intercellular mycelium and penetration of haustoria. ×900. C. Group of epidermal and cortical cells of resistant host showing formation of small lobed haustorium. ×750. D. Giant cell from pathological xylem of resistant stem. ×700. E. Tracheid cells of susceptible host showing haustoria encased in lignified callosities. ×930. F. Epidermal cell of susceptible host showing germ tube which has penetrated the cuticle and is growing over the surface of the epidermal cell. ×950. G. Portion of wall of epidermal cell of resistant host showing the path of penetration of the germ tube through the cuticle. ×1900.

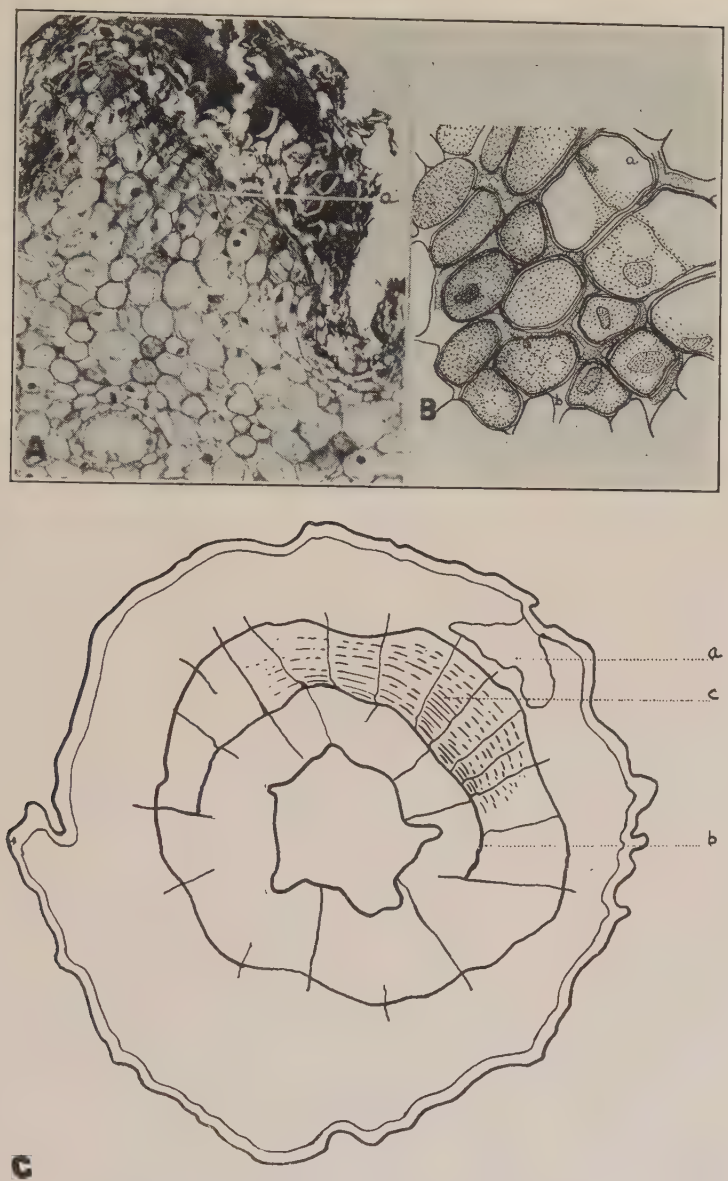

FIG. 4. A. Portion of transverse section of resistant stem of *Pinus sylvestris* one month after inoculation. a. Phellogen below necrotic area showing stimulation. B. Portion of cortical region of susceptible host directly beneath epidermis. The granular cells at the left are those first attacked by the fungus after penetration. These cells contain much tannin. Note stunted haustoria in cells a and b. ×300. C. Diagram of transverse section of resistant stem three months after inoculation. a. Necrotic area in cortex and phloem where cells have died following fungus invasion. b. Pseudo-annual ring formed by inhibited cambium derivatives. c. Region of tangentially elongated tracheids, bending of rays, and excessive formation of secondary rays. ×35.

directly after penetration of the epidermis. The cortical cells do not become filled with tannin and, in fact, appear perfectly normal (Fig. 3, B). In such cases there would seem to be an immediate adjustment of parasite and host without the preliminary hesitation and gradual adjustment as described above.

During the latter part of the growing season in which infection occurred, or the beginning of the following season, this semimutualistic balance becomes somewhat upset in that there is a decided stimulation of the host cells resulting in gall formation. Still, in the developing gall, little change can be noted in recently parasitized cells. A large number of the cells in the developing gall become gorged with starch and may be penetrated by haustoria. Starch is not confined to normal storage tissues, but may be found even in thin-walled tracheid cells. Some cells in the gall have undergone degeneration, and often cells adjacent to these contain much tannin.

It is notable that in reaction type A the mycelium may spread unhindered by any cork barrier. By the end of the season in which infection occurred, the mycelium has advanced to the cambium. Haustoria penetrate many of the newly formed nonlignified xylem elements. As these cells mature the haustoria become surrounded by heavy layers of cell-wall material (Fig. 3, E), forming structures similar to the lignitubers described by Fellows (17); these protuberances usually stain in the same manner as lignified membranes. The haustoria embedded within these protuberances appear to remain alive, but have not been observed to break through this heavy encasement. Much more delicate callosities are apparently formed about haustoria in invaded cortex, phloem, and ray cells.

Reaction Type B. In partially resistant plants the early stage of infection corresponds to that in susceptible plants in that tannin accumulates in cells adjacent to the mycelium. Within a few weeks after inoculation, it can be noted that a group of host cells has died. The cell walls are thickened, and the cells often contain brownish or yellowish globules. Mycelium can rarely be seen within or between these dead cells, but is abundant in adjoining areas, where it forms many haustoria. A suberized layer, usually 3 to 4 cells wide, is formed enclosing this necrotic area, as well as the infected tissue adjacent to it. The mycelium advances to this layer of wound cork and in many cases breaks through it, infecting the healthy tissue beyond. As a result, some of these infected cells die, a second cork layer may be formed, and the progress of the mycelium again checked. Three such alternating layers of necrotic tissue and cork have been observed in some twigs. When the mycelium finally gains a foothold and is no longer checked, a gall may be developed, but it is often

aborted or atypical. Usually, however, the mycelium seems to degenerate, becoming unable to break through the second or third cork barrier, and finally disintegrates. As the twig continues to grow, the bark is split and there is an exfoliation of the necrotic areas. This may continue for 2 or 3 seasons and may sometimes be accompanied by a generalized swelling in this area. This would appear to be due to the stimulation of the cambium in a manner probably similar to that described under reaction C.

Reaction type C. Sections through the discolored infection spots commonly found on all trees after artificial or natural inoculation, but frequently the only symptom of infection in the resistant trees, show small necrotic areas in which often no mycelium is evident. As soon after penetration as the fungus comes in contact with the subepidermal cells, these cells respond by becoming intensely granular with tannin, as described above. They also develop thickened cell walls, at least on the side near the mycelium. An occasional haustorium (Fig. 3, C) may penetrate these cells before this response occurs. As the mycelium makes feeble progress, the cortex cells first attacked continue to develop even thicker walls, and the cells, instead of remaining granular, become filled with large globules that, in unstained sections, appear yellowish brown. Microchemical tests would indicate that some of these globules are composed of gum, and others of oil or fat, both types being in some way associated with tannin. Haustoria in such collapsed cells occasionally retain nuclei and appear normal for a short time before their death and disintegration.

Sections examined one month after inoculation, at a time when these infection spots are visible macroscopically, show definite necrotic areas, often extending as far as the phellogen (Fig. 4, A). Many cells are collapsed, the majority contain the globules described above, the cell walls are thick and often lignified, and the entire area takes a very heavy stain with safranin. The cells adjacent to such areas show a stimulation in that the nucleus is increased in size and the cells show an increased staining ability. In the nearby phellogen regions, tangential division of the cells has occurred to a greater extent than in the normal portions of this tissue. A search for mycelium or haustoria among this group of dead cells has been uniformly unsuccessful. These necrotic areas, then, may be considered similar to those reported in various resistant hosts infected with rusts (3, 24, 25, 33, 40). These regions of necrotic tissue are soon sloughed off as the twig continues to develop.

In these resistant twigs, as mentioned above, an occasional swelling may form, as though galls were to be produced. In the development of such areas, it would appear that there is a delayed response in the first-attacked cortex cells. The formation of tannins, the thickening of cell walls, and

finally the development of globules and the collapse of the cells do not accompany the early infection. Consequently some of the hyphae may progress to other normal cells before they are checked by the death of the first-invaded tissue. In this way the mycelium is able to reach the cambium during the first growing season. The hyphae are by no means abundant, but tend to be fragmentary and can be seen only with difficulty. During the progress of the parasite, the host cells directly back of and adjacent to the advancing hyphae have died, and there is consequently formed a wedge-shape area of necrotic tissue extending from the epidermis to the cambium (Fig. 4, C). The majority of the cells in this area are dead. Globular contents of cells and excessively thickened cell walls are evident. Some cells that have not died possess very large nuclei and may contain much starch or oil. This wedge-shape area is separated from the normal cortex and phloem by a layer of wound cork. Adjacent to this the cells show the result of stimulation in that they stain much more heavily than similar normal cells, and are often gorged with starch. Early in the growing season following that in which infection occurred, a layer of sclerenchyma is formed adjacent to the necrotic area and directly external to the wound cork. Any mycelium that may be distinguishable at this time appears to be dead and disintegrating.

Cambial development during the latter part of the first season results in the formation of extremely pathological tissue. A portion of the cambium, often embracing half of its circumference around the twig, may be almost completely inhibited in development (Fig. 4, C), while the rest of the cambium is developing normally. The few divisions that may occur in these inhibited cells yield derivatives that fail to mature but appear to collapse and die very soon after formation. Later in the same growing season, however, this inhibited tissue may become greatly stimulated. Individual cambial cells increase in size, especially in a tangential direction, often becoming 4 to 6 times as long as normal cells. The tracheids, as well as phloem elements derived from such cambium cells, are also very much elongated tangentially. The stimulus that brings about this pathological condition often continues to act during the second season after inoculation, although it appears to be greatly weakened, for the cambium and its derivatives gradually become normal.

The xylem elements, formed just prior to and during the attack of the fungus on the cambium, some of which may be invaded by haustoria, do not complete their differentiation, but in many cases remain thin walled, retain cytoplasm and nuclei, and may be excessively pitted. Quantities of starch and oil may be stored in such juvenile xylem cells. Giant cells are frequently produced (Fig. 3, D) and it is not uncommon to find the

formation of tylosis-like cells extending through a pit in one tracheid into another. According to Chrysler (7) this phenomenon in pine is a result of wounding. Resin ducts in such areas are filled with tylosis-like cells in which starch may be stored. Rays developing through such abnormal areas are composed of abnormally large cells in which also much starch and oil may be stored. There is a very noticeable bending of these rays (Fig. 4, C), the change in direction of growth occurring at the point where the cambium is inhibited and its derivatives killed.

The study of sections through infected areas, cut during the latter part of the third year after inoculation, usually shows that the stem tissues have recovered from the effect of the stimulus. The juvenile tracheids and the layer of dead-inhibited-cambium derivatives in the first-year xylem compose what appears like a pseudo-annual ring extending half way or more around the stem (Fig. 4, C). External to this may be a considerable area of tangentially elongated or abnormally large tracheids. The tracheids of the late second-season and entire third-season growth have become gradually shorter tangentially, and those nearest the cambium are entirely normal. The bending of the rays at the position of the pseudo-annual ring is evident throughout the abnormal portion of the stem. An unusually large number of secondary rays arise as the tissues become more nearly normal (Fig. 4, C). The cambium as well as the more recently developed phloem tissues appear normal. The wedge-shape necrotic area has become completely surrounded by wound cork and sclerenchyma. The entire area stains heavily with safranin, and many of the thickened cell walls appear to be lignified. During the next growing season, this area is gradually crowded out nearer and nearer to the exterior of the twig. Portions of it are gradually sloughed off and finally the whole area is thus removed. The study of the progress of these later stages is often complicated by the fact that there may be a secondary infection, possibly by a wood-rotting fungus.

From the standpoint of pathological anatomy, the juvenile tracheids that retain cytoplasm and nuclei and are checked in their differentiation typify a hypoplasia (23). On the other hand, the formation of enlarged tracheids, giants cells, and tyloses in tracheids and resin ducts would represent a hyperplastic condition. The entire area of pathological xylem can be considered as wound wood.

In view of the large amount of pathological tissue formed in these stems, there might be some doubt about referring to them as resistant. However, since the fungus is definitely checked in these tissues, never comes to fruition, and furthermore does not sufficiently stimulate the tissues to produce a gall, these stems may well be considered resistant.

PHYSIOLOGICAL STUDIES

Chemical Analyses

Collections of twigs of the current season's growth were made from 12 resistant trees varying in age from 8 to 15 years. Each tree had been artificially inoculated for several successive years, but had failed to produce any galls, the reaction to infection being always of type C. These trees were typical of the entire group under consideration, as they were chosen from the different plots, some growing in partial shade and others in full sunlight, some growing on hillsides and others on level ground. Collections also were made from 12 susceptible trees, 8 to 15 years old and growing under conditions similar to those described above. Each tree had numerous mature galls. These collections from susceptible trees were further divided into one group consisting of twigs without galls and into another group containing only galls.

The selection of samples for analysis was made by dividing each twig into several portions and making a random choice of these parts after they had been thoroughly mixed.

Analyses have been made particularly for tannin,[6] potassium, and calcium.[7] The results expressed in per cent by weight are as follows:

	Resistant	Susceptible	Galls
Moisture-free basis			
Soluble solids	12.9	13.8	16.3
Non-tannins	8.7	9.9	11.2
Tannin	4.2	3.9	5.1
As plant ash			
CO_2 and C free ash	1.2	0.93	
CaO	29.86	33.32	
K_2O	41.40	18.09	

Perhaps the two points of significance in these results are the great excess of potassium in the resistant plants and the higher content in the galls of both tannin and other soluble solids. It is recognized that these results should not be given undue weight, as they represent but one determination. Yet the striking difference in potassium content of these two carefully chosen samples may be of considerable significance. The rôle of potassium in the plant (27) is thought to be closely connected with the synthesis of carbohydrates and probably of proteins and oils also. If,

[6] The tannin analysis was made by the Division of Industrial Farm Products, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

[7] The potassium and calcium analysis was made by Mr. H. J. Hallowell, Consulting Chemist, Philadelphia, Pa.

then, resistance may have some nutritional basis, this potassium relationship may be fundamentally related to it. It has been repeatedly shown (13, 30, 31, 32), that plants grown with a lack of potassium are more susceptible to disease than those grown with sufficient potassium.

Analyses of samples of soil from both surface and subsoil[8] collected from the base of three resistant trees and three susceptible trees growing under the same conditions in very close proximity have failed to reveal any significant difference in potassium or calcium content. The following results are based on air-dried soil:

Trees	Sample	Potassium %	Calcium %
Resistant	Surface soil	1.24	0.60
	Subsoil	2.29	0.80
	Surface soil	2.20	0.60
	Subsoil	2.29	0.73
	Surface soil	2.29	0.52
	Subsoil	2.49	0.47
Susceptible	Surface soil	2.65	0.63
	Subsoil	2.53	0.94
	Surface soil	2.61	0.58
	Subsoil	2.69	0.74
	Surface soil	2.37	0.62
	Subsoil	2.52	0.68

Relation of Cell Sap to Growth of the Fungus

In a further attempt to note some physiological difference between susceptible and resistant trees, a study was made of the effect on the fungus of juices extracted as described above from noninfected susceptible and resistant trees. Since it has not been found possible to produce mycelium by culturing rusts on any sort of culture medium, the criterion of fungus growth could be only spore germination and a comparatively slight growth of the germ tube. The spores were sown in drops of the liquids on depression slides and kept at room temperature. In view of the fact that tannin has been found in some cases to be a deterrent to fungus growth, the effect of various strengths of tannin dissolved in distilled water has been similarly studied.

The results of these studies are summarized in table 1. It will be noted that the juices from susceptible and resistant trees have approximately the same relationship to the growth of the fungus. The resistant juice does not show the deterring effect that might be expected, but even would

[8] The soil analyses have been made by the Division of Soil Fertility, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

appear to increase the percentage of germination. The results of the study with tannin indicate conclusions similar to those reached by Cook and Taubenhaus (10), *i.e.*, that low percentages of tannin solution may stimulate fungus growth, perhaps acting as food, but that higher percentages may inhibit or prevent growth.

Physico-chemical Constants of Cell Sap

Samples of juices freshly expressed from resistant and susceptible twigs as described above were used to determine osmotic pressure by means of the Beckman thermometer, and hydrogen-ion concentration by means of the potentiometer. The following constants have been secured:

Sample	pH	Osmotic pressure in atmospheres
Resistant	3.84	13.60
Susceptible	3.76	13.69

The rather close correspondence of these figures would indicate very little difference between the resistant and susceptible trees in these respects.

Injection Experiments

The formation of abnormal tissues in the resistant twigs, even after the mycelium had been checked and had disintegrated, would indicate that the stimulation may be due to some chemical formed by the disintegrating mycelium or host cells. Two sets of injection experiments have been performed to attempt to induce such abnormal changes by chemical means. An extract was made of mature galls by grinding them with sand and distilled water and filtering the liquid through a Seitz filter. A portion of the filtrate was shaken with aluminum hydroxide and refiltered, much of its brownish color being removed by this process. Another extract was prepared by germinating spores until the germ tubes had reached their maximum length. They were then frozen in distilled water so that the germ tubes were ruptured and the liquid was then filtered.

These three extracts were introduced into the plant tissues, both by hypodermic and capillary injection.

In the same manner 1 per cent and 0.1 per cent solutions of oxalic acid were injected into twigs. Controls of injections of distilled water and simple needle punctures were made on several trees.

During the course of 3 months some of the injected twigs developed slight swellings and resinosis. Collections were made at intervals of 1 month for 3 months, and the tissues prepared and stained as described

TABLE 1.—*Germination and growth of germ tube of spores of Peridermium sp. sown in various dilutions of tannin and in juices extracted from susceptible and resistant trees*

Medium	No. of spores examined	No. of spores germinated after 4 hours	Germination per cent	Average length of 10 longest germ tubes after 4 hours μ	Average length of 10 longest germ tubes after 18 hours μ
Juice extracted from uninfected resistant trees	1135	88	7.8	57	192
Juice extracted from uninfected susceptible trees	1143	32	2.8	68	192.5
Tannic acid— 1%	780	0	.....	.....	.....
" " 0.1%	316	0	.....	.....	.....
" " .075%	1060	185	17.4	105	.....
" " .05%	565	53	9.4	88.2	.....
" " .025%	1200	77	6.4	74.2	.....
" " .01%	900	40	4.4	30.0	225
Distilled water	360	0	.....	.....	.....
Tap water	800	20	2.5	83.8	210

above. A comparative study of the control twigs and those injected with chemicals has revealed no cell or tissue abnormality other than could be attributed to wounding. Adjacent to the wound caused by the needle or tube several cell layers had collapsed and died. Internal to this area a cicatrice layer had formed about the entire wound. For several cell layers internal to this there was a decided stimulation of cells in that they stained more heavily than similar normal cells and the nuclei were often larger than in normal cells. There appeared to be no stimulation of the cambium due to the diffusion of the extracts or acid into this tissue.

DISCUSSION

Studies of resistance among plants to parasitic organisms have revealed the fact that this resistance may be morphological, physiological, or functional in nature. It has further been shown that the resistance of a plant may depend, not on a single phase, but may perhaps be determined by all phases.

In the present study attempts have been made to correlate resistance with some morphological feature or with some physiological aspect of the

plant and, in a study of the reactions of the plants to inoculation, to note any differences that might help to explain resistance.

Twigs from noninfected resistant and susceptible trees have shown no histological or microchemical differences that might account for the different reactions to invasion by the rust. In relation to the physiological aspect, the only outstanding difference noted is the potassium content of the noninfected twigs. Perhaps too much significance should not be placed upon this fact. Yet, in view of the generally accepted rôle of potassium in the plant as a catalyst in carbohydrate formation, the relationship of the excess of potassium in resistant trees to their resistance might well be investigated further.

It is, however, from a study of the reactions of the plants to inoculation that most can be learned in this case about the nature of resistance. The fungus in one plant may progress unimpeded and establish itself in an almost mutualistic relationship with the host, while in the tissues of another plant, or in the tissues of another portion of the same plant, the host cells may be killed almost immediately and the fungus partially or completely checked in its progress. The cause of these markedly different host reactions is not easily determined.

It is well first to consider how the results of this study may be correlated with the starvation hypothesis suggested by Ward (37). The complete checking of the parasite by the collapse and death of invaded host tissue might be considered to be the result of the inability of the fungus to secure food from its living host cells, and its further inability to adapt itself to any saprophytic mode of life.

The occasional spread of the mycelium from such necrotic areas, especially when the host reaction has been a mild or gradual one, would appear to depend upon the vigor of the fungus. This vigor is without doubt determined by the amount of food the hyphae are able to secure before the necrosis sets in. This food may be transferred to the advancing hyphal tips, which are thus able to grow beyond the dead tissue areas. If the fungus in its food absorption can keep ahead of the host necrosis, it may be able to advance even to the cambium during the first season. If, however, the host necrosis becomes more rapid, the mycelium may lose its vigor and degenerate since it is surrounded by dead host tissue. In the susceptible host the cells would appear to be stimulated to acquire additional food material for the use of the fungus. The fungus thus increases in vigor and advances unhampered through the tissues.

True[9] has noted that even in the most susceptible Scotch pine trees very heavy inoculations with this same species of Peridermium may result in no

[9] True, R. P. Gall development on *Pinus sylvestris* attacked by the Woodgate Peridermium and morphology of the parasite. In manuscript.

gall formation, but in the cracking of bark characteristic of reaction type B. This fact might also be explained on a nutritional basis. The numerous mycelia spreading from several infection centers may defeat each other in their struggle to secure food from the host and none may be able to establish itself.

The same explanation may be given for the failure of many infection centers to develop in the susceptible host, although from one such center the mycelium spreads and produces a gall. The mycelium that is able to establish an immediate congenial relationship with the host cells would appear to secure almost a monopoly on the food supply.

As Wellensiek (40) has pointed out, starvation may result not only from the entire lack of individual food substances, but may be brought about by only a slight variation in the quantity of some food material. The apparent difference in potassium content between suceptible and resistant twigs may indicate some carbohydrate excess or deficiency, as suggested by Mains (24).

The failure to demonstrate by injection experiments the presence of any toxic material in the fungus should not invalidate an assumption that toxin production may take place in the living cell invaded by haustoria. Allen (1) has considered that such an assumption may best account for resistance in wheat. There is evidence that the death of host cells precedes the death of the invading haustoria; similar evidence has been obtained by Allen (1) and Stakman (33). It would thus appear possible that a toxic substance released by the fungus is responsible for the death of the host cells. The disintegrating cell contents in turn may release materials harmful to the haustorium. It also is conceivable that in these poisoned host cells the food available for the fungus may be rendered unavailable, and the haustorium will soon die from starvation. On the other hand, in reaction type A the toxin produces no visible injury, but stimulates the cells to increase in size and number. It would thus appear that cells showing the resistant reaction are hypersensitive to the excretion of the fungus.

It has been noted that a layer of cork cells develops about the infected tissue in both reaction types B and C. This is later followed by the formation of a continuous layer of sclerenchyma cells about this region. This corky layer might at first be considered to have some definite relation to resistance. However, the same type of tissue may be formed as a reaction to mechanical wounding. Invaded tissues that respond by collapsing and becoming necrotic are in reality wounded tissues and function as a pseudocicatrice layer (42). The continuous layer of cork may then be considered a cicatrice layer and thus to represent solely a response to wounding. The usual lack of cork development in those infected areas in the susceptible host from which galls develop is apparently due to the fact that here the

host cells are not injured or wounded. Only when the fungus is low in vigor and apparently unable to secure sufficient nourishment is it checked by this cork layer. Thus this layer should be considered only meagerly related to resistance.

The accumulation of tannin in those cortical cells first attacked by the fungus has been found accompanied by at least a partial checking of the mycelium. While tannin also has been observed in abundance in tissues of wounded control twigs, it would seem to have some bearing upon resistance in this case. As has been pointed out, the mycelium may spread rapidly in the susceptible host without any accumulation of tannin in the host cells. In other susceptible twigs the mycelium makes slow progress until it grows beyond the tannin-containing tissues. In some resistant, as well as some susceptible twigs, the mycelium makes no progress beyond the tissues in which tannin accumulates. It is quite probable that other factors besides tannin accumulation are involved in this partial or complete retarding of the fungus. It is not clear in just what manner tannin may help to bring about this reaction. Haustoria growing into such tannin-filled cells are not usually killed until the cell itself has died. The study of spore germination and growth of germ-tubes in various percentages of tannin might indicate that the action upon the parasite is somewhat toxic.

This accumulation of tannin in host cells might well be considered as representing the "local immunity" suggested by Dufrénoy (14) for similar accumulations of phenolic compounds.

SUMMARY

A comparative study of stem tissues and extractives of *Pinus sylvestris* trees susceptible and resistant to a species of Peridermium has been made in order to determine the nature of resistance to this parasitic fungus.

No correlation has been found between any morphological feature of the noninfected host and the resistance to infection.

Three general types of reaction to inoculation may be distinguished: (1) typical gall formation on the susceptible host; (2) cracking of bark and slight resinosis sometimes followed by the formation of atypical galls on the partially resistant host; (3) formation of small necrotic areas on twigs followed sometimes by swellings that do not increase in size after the first or second year but are gradually sloughed off.

In the most susceptible plants the mycelium may actively grow in the host and live in something of a mutualistic relationship. This results in stimulation of living cells and the formation of a gall.

In the most resistant trees the host cells are killed immediately after invasion. The fungus also soon dies and the necrotic area is sloughed off.

Often, in both resistant and susceptible plants, the first-invaded cells

may become filled with tannin. This reaction may represent a local immunity.

In those resistant trees that develop swellings, mycelium grows to the cambium and then dies. Areas of necrotic tissue extend from epidermis to cambium. The cambium becomes stimulated to form quantities of wound wood including tangentially enlarged tracheids, giant cells, and tyloses in tracheids and resin ducts.

In the resistant reaction, the necrotic area becomes surrounded by cork and later by sclerenchyma. This probably represents a normal reaction to the wounding of cells, and may play a small part in resistance.

No correlation has been observed between the osmotic pressure or hydrogen-ion concentration of cell sap and resistance.

A greater amount of potassium has been found in noninfected resistant stems than in susceptible stems. This is thought to have some possible relationship to resistance on the basis of nutrition.

It is considered that the liberation of toxin into cells by the haustoria may cause the death of the hypersensitive cells of the resistant host. The fungus is thus deprived of its food supply and soon dies. In the susceptible host, however, toxin does not kill the cells at once but simply stimulates them to gall formation.

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